

**A STUDY OF FECAL MICROBIOTATransplantation (FMT) FOR THE TREATMENT OF RECURRENT
C. DIFFICILE ASSOCIATED DIARRHEA (RCDAD) VIA RETENTION ENEMA OR ORAL ROUTE**

NCT02449174

Version Date: 07/13/2017

Clinical Study Protocol

A STUDY OF FECAL MICROBIOTATransplantation (FMT) FOR THE TREATMENT OF RECURRENT *C. DIFFICILE* ASSOCIATED DIARRHEA (RCDAD) VIA RETENTION ENEMA OR ORAL ROUTE

Study Number: HSC-SPH-14-0020

Investigational Product

Frozen Healthy Donor Intestinal Bacteria
Lyophilized Healthy Donor Intestinal Bacteria

Sponsor

The University of Texas Health Science Center

1200 Pressler Room 741

Houston, TX 77030

Principle Investigators:

Dr. Herbert L. DuPont – Co-director responsible for review and evaluation of information relevant to the safety and efficacy of the study product
Professor and Director, Center for Infectious Diseases, UT-SPH, 1200 Pressler Room 737, Houston, TX 77030
Ph: 713 500 9366, Email: Herbert.l.dupont@uth.tmc.edu

Dr. Zhi-Dong Jiang – Co-director responsible for monitoring the conduct and progress of the clinical investigations and responsible for screening of donors and preparation of FMT for administration
Associate Professor and Director, Enteric Pathogens Diagnostic Laboratory, Center for Infectious Diseases
UT-SPH, 1200 Pressler Room 741, Houston, TX 77030
Ph: 713 500 9371, Email: zhi-dong.jiang@uth.tmc.edu

Dr. Andrew W. DuPont – responsible for monitoring the conduct and progress of the clinical investigations at Memorial Hermann Hospital study site
Associate Professor, Internal Medicine, Gastroenterology, 6431 Fannin, Houston, TX 77030-1503
Ph: (713) 500-6687, Email: andrew.w.dupont@uth.tmc.edu

Dr. Ned Snyder – responsible for monitoring the conduct and progress of the clinical investigations at Kelsey-Seybold Clinic study site
Chief of Gastroenterology, Internal Medicine, Gastroenterology, 2727 West Holcombe Blvd.
Houston, Texas 77025
Ph: (713) 442-0000, Email: Ned.snyder@Kelsey-Seybold.com

TABLE OF CONTENTS

SYNOPSIS..... 4

1. INTRODUCTION 8

1.1 Background 8

1.2 Summary of Fecal Microbiota Transplantation from Publications and Our Previous Study (HSC-SPH-13-0119) 8

2. OBJECTIVE 9

3. STUDY DESIGN..... 9

3.1 Study Structure 10

3.2 Study Population..... 10

3.3 Study Procedures 11

3.3.1 Informed consent..... 11

3.3.2 Identification number 11

3.3.3 Inclusion and exclusion criteria..... 11

4. METHODOLOGIES 12

4.1 Recipient 12

4.1.1 Pre-screening 12

4.1.2 One-Two Days Before FMT 13

4.1.3 Day before FMT..... 13

4.1.4 Day of FMT 13

4.1.5 Approximately One day after FMT..... 14

4.1.7 Approximately Seven days after FMT 15

4.1.8 Approximately Fourteen days after FMT 15

4.1.9 Approximately Thirty days after FMT 16

4.1.10 Approximately Sixty days after FMT 16

4.1.11 Approximately Ninety days after FMT 17

4.1.12 Safety data 17

4.2 Donor 17

4.2.1 Pre-screening 18

4.2.2 One day before donation..... 18

| | | |
|-------|--|----|
| 4.2.3 | Day of donation – preparation, storage and shelf life | 18 |
| 5. | EVALUATION OF SAFETY | 20 |
| 5.1 | Primary Safety Endpoints..... | 20 |
| 5.1.1 | Medical conditions..... | 20 |
| 5.1.2 | Monitoring for safety | 20 |
| 5.1.3 | Pre-existing signs and symptoms and medical conditions..... | 22 |
| 5.1.4 | Progression of underlying conditions as an adverse experience..... | 22 |
| 5.1.5 | Recording and documenting adverse experience..... | 22 |
| 5.1.6 | Investigator reporting of serious adverse experience | 23 |
| 5.1.7 | Notification of post-study serious adverse experience | 23 |
| 5.2 | Secondary Efficacy Endpoint..... | 23 |
| 6. | DATA COLLECTION AND ANALYSIS..... | 24 |
| 6.1 | Recording of Data | 24 |
| 6.2 | Statistical Methods | 24 |
| 6.2.1 | General considerations | 24 |
| 6.2.2 | Power calculations | 24 |
| 6.2.3 | Analysis populations | 24 |
| 6.2.4 | Randomization procedures..... | 24 |
| 6.2.5 | Analyses of safety..... | 25 |
| 6.2.6 | Secondary efficacy analyses..... | 25 |
| 7 | PROTECTING PRIVACY | 25 |
| 8 | CONTACTS..... | 25 |
| 9 | REFERENCES..... | 26 |

HSC-SPH-13-0119

SYNOPSIS

| | |
|--------------------------------|---|
| TITLE | A Study of Fecal Microbiota Transplantation (FMT) for the treatment of Recurrent <i>C. Difficile</i> Associated Diarrhea (CDAD) via Retention Enema or Oral Route |
| PROTOCOL | HSC-SPH-14-0020 |
| SETTINGS | Attending physicians of recipients/subjects will recommend the FMT and make clinical decisions related to overall health of his/her subjects. All subjects undergoing therapy will be handled as outpatients at either Baylor St. Luke's Medical Center, the Digestive Disease Center at Memorial Hermann, Kelsey-Seybold Clinic – Main Campus on Holcombe Blvd. or University of Texas School of Public Health, Center for Infectious Diseases. |
| OBJECTIVE | To evaluate the safety and preliminary efficacy of frozen intestinal bacteria from a healthy donor given by retention enema or lyophilized intestinal bacteria given orally in capsules for therapy in subjects with recurrent <i>C. difficile</i> associated diarrhea (RCDAD) |
| STUDY DESIGN | Allocation: Randomized Endpoints: Safety, preliminary efficacy in preventing future bouts of CDAD and improvement in intestinal flora diversity Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment |
| INCLUSION CRITERIA - RECIPIENT | All recipients <ol style="list-style-type: none"> 1. Male and female subjects 18 years of age or older 2. Sexually active female subjects of child-bearing potential must agree to use an effective method of birth control during the treatment and follow-up period 3. Required to sign an informed consent form 4. Referred by subjects attending physician who will provide non-transplant care for the subject 5. Able to follow study procedures and follow-ups 6. Diagnosed by medical history of ≥ 3 recurrent CDAD bouts (RCDAD) in outpatients or ≥ 2 bouts of RCDAD in an inpatient with ≥ 2 positive fecal tests for <i>C. difficile</i> toxin 7. Received at least one course of adequate antibiotic therapy for CDAD (≥ 10 days of vancomycin or metronidazole or fidaxomicin) since last bout of CDAD |
| INCLUSION CRITERIA - DONOR | <ol style="list-style-type: none"> 1. Able to provide and sign informed consent 2. Able to complete and sign the donor questionnaire 3. Able to adhere to FMT stool collection requirements |
| EXCLUSION CRITERIA - RECIPIENT | <ol style="list-style-type: none"> 1. Subjects with known neutropenia with absolute neutrophil count $<0.5 \times 10^9/L$ 2. Evidence of toxic megacolon, fulminant colitis, gastrointestinal perforation, ileus, abdominal distension, lack of bowel sounds, fever, or hypotension 3. Known peripheral white blood cell count $> 15.0 \times 10^9/L$ or temperature $> 38.0^\circ C$ 4. Diarrhea due to definable non-CDAD pathogen 5. Post total colectomy or presence of a colostomy 6. Unable to tolerate FMT via enema for any reason or to take capsules orally 7. Requiring systemic <u>non-<i>C. difficile</i></u> antibiotic therapy at the time of FMT |

| | |
|----------------------------|---|
| | <ol style="list-style-type: none"> 8. Actively taking <i>Saccharomyces boulardii</i> or other probiotic at the time of FMT 9. Need for continuing use of oral vancomycin, oral or IV metronidazole, fidaxomicin, rifaximin or nitazoxanide at the time of FMT and after FMT 10. Severe underlying disease such that the patient is not expected to survive for one or more years or unstable medical condition requiring daily change in treatments |
| EXCLUSION CRITERIA - DONOR | <ol style="list-style-type: none"> 1. Tested positive for any of variables mentioned in the section of Screening of Donor 2. History of any type of active cancer or autoimmune disease 3. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire 4. History of gastrointestinal disorder, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or active diarrhea 5. Antibiotic use or any systemic immunosuppressive agents in the 3 months prior to stool donation 6. Current or previous medical or psychosocial condition 7. Body mass index over 30 8. Presence of known diabetes mellitus 9. Travel to a developing country in past 3 months |
| SCREENING OF DONOR | <p>Stool will be tested for following enteric pathogens:</p> <ol style="list-style-type: none"> 1. <i>Shigella</i> 2. <i>Salmonella</i> 3. <i>Campylobacter</i> 4. Shiga-toxin Producing <i>Escherichia coli</i> 5. <i>Clostridium difficile</i> Toxin A/B 6. Norovirus <p>Blood sample from donor will be tested for following items:</p> <ol style="list-style-type: none"> 1. Hepatitis B core antibody 2. Hepatitis B surface antigen 3. Hepatitis C virus antibody 4. HIV-1 and HIV-2 antibody 5. Anti-HTLV I/II 6. Serologic test for syphilis |
| DONOR STOOL PREPARATION | <p>Healthy/screened donors will donate multiple stools of $\geq 150\text{g}$ each used for both intestinal bacteria forms – frozen to be given by enema and lyophilized to be given by capsules) at the University of Texas School of Public Health. Stool will be mixed with sterilized 0.9% NaCl (1:5 dilution for frozen bacteria and 1:2 dilution for lyophilization), then mix above solution in a homogenizer mixer with sterilized bag (Stomacher). All the products will be labeled and kept at -80°C for up to 12 months.</p> |
| STUDY SCHEDULE | <p>After a consent form is signed by the potential FMT recipient, a pre-FMT stool will be collected to test for <i>C. difficile</i> toxins and an aliquot (2 mL) will be stored at -80°C for future studies. One day before FMT, subjects will be prepared for FMT by following a clear liquid diet the entire day followed at $\sim 6:00\text{ pm}$ by taking of GoLytely or other suitable bowel prep to prepare colon for FMT. All subjects will take 4 mg (2 capsules of loperamide (Imodium) in the morning of FMT, or if missed this dose will be taken after FMT. After the FMT is complete the subject may resume a normal diet unless they are on a special diet for other medical condition. The method of FMT treatment is randomized and the subject will receive either a retention enema or oral capsules. Subject follow up is planned on or</p> |

| | |
|--|---|
| | <p>about days 1, 7, 14, 30, 60, 90 and 6 months after FMT to monitor any adverse experience and/or to obtain the name and dosage of any medication taken. If possible, a fresh stool sample will be collected from the recipient on or about days 2, 7, 14, 30, 60 and 90, or at any time the subject reports onset of diarrhea during the first 90 days after FMT. The stool will be tested for <i>C. difficile</i> toxins and an aliquot (2 mL) stored at -80°C for future studies.</p> <p><u>Treatment Failure and Retreatment:</u></p> <p>Treatment failure is defined as experiencing RCDAD within 60 days after FMT. CDAD is defined as passage of ≥ 3 watery stools per 24-hour period for two consecutive days together with a positive fecal test for <i>C. difficile</i> toxin. Two additional treatments (total of three treatments) may be provided for treatment failures providing the criteria above are met:</p> <p>If treatment failure recurs within 60 days after the FMT treatment, it will be recommended to the study subjects' physician that the subject be treated with 14-21 days of oral vancomycin or fidaxomicin. The subject would then be offered subsequent FMT treatment 1-2 days after stopping the antibiotic and any probiotics self-administered. The subject would receive same route of FMT as previous treatment using different donor stool, at no additional charge to the subject.</p> <p>With subsequent treatment failure this sequence of treatment with vancomycin or fidaxomicin followed by a third FMT would be made available if the subject recurred after the second FMT. After this third FMT, no additional FMT will be performed and subject will be considered a FMT failure. The subject will be referred back to their physician to resume their patient care with a recommendation that FMT not be further pursued in their management.</p> |
| DURATION OF FOLLOWUP | The duration of study participation for each patient is approximately 94 days. We will attempt to follow up subjects on or about 6 months after FMT in order to monitor any health effects for safety. |
| STUDY POPULATION | <p>Study subjects will be identified and receive FMT beginning September 2014.</p> <p><u>Recipient:</u> Up to 100 adult male or female subjects with history of ≥ 3 RCDAD as outpatients or ≥ 2 RCDAD hospital inpatients in the previous year</p> <p><u>Donor:</u> Up to 15 donors will be identified to provide multiple stools of ≥ 150 g of stool each</p> |
| STUDY AGENT, DOSE & MODE OF ADMINISTRATION | Processed intestinal bacteria from a screened healthy donor will be administered later either as frozen FMT by retention enema or lyophilized FMT (PRIM-DJ2727) to be given by the oral route. |
| EFFICACY EVALUATION | <ul style="list-style-type: none"> Primary Endpoint: Safety of FMT (frozen product or lyophilized capsules) Secondary Endpoint: Prevention of subsequent bouts of CDAD in 60 days post FMT. |
| SAFETY EVALUATION | Safety will be assessed by evaluating any unfavorable or unintended signs, symptoms, or disease temporally associated with FMT procedure and will be recorded as an adverse |

| | |
|--|--|
| | experience. Data will also be collected about the frequency and/or severity of the signs or symptoms of enteric infection (defined as fever, moderate to severe gas/flatulence, nausea, vomiting, abdominal cramps or pain, rectal tenesmus, or defecation urgency). We will attempt to follow up subjects on or about 6 months after FMT to obtain information about recurrence or any adverse effects. |
|--|--|

1. INTRODUCTION

1.1 Background

During the past several years, *C. difficile* associated diarrhea (CDAD) has become more frequent and severe, more refractory to standard therapy, with predictable recurrence rates of 25% after standard therapy [1, 2]. It is now accepted that the disruption of the normal balance of colonic microbiota secondary to antibiotic use facilitates the development of CDAD and improvements in the diversity and quantity of flora is associated with recovery of infection and prevention of disease recurrence. Studies have shown that subjects with recurrent CDAD (RCDAD) have decreased diversity of colonic flora with absolute reductions in counts of anaerobic species of *Bacteroidetes* and *Firmicutes* in their stool compared to subjects recovering from single episodes of CDAD [3]. Numerous case reports and retrospective case series have demonstrated the benefit of intestinal microbiota transplantation (FMT) in subjects with severe or RCDAD with cure rates over 90% [4-8], making FMT standard therapy when multiple recurrences are seen [9]. FMT involves administration of a suspension of intestinal bacteria obtained from a healthy individual into the GI tract of a patient with RCDAD to promote normalization of flora [5].

1.2 Summary of Fecal Microbiota Transplantation from Publications and Our Previous Study (HSC-SPH-13-0119)

This study compliments our currently approved FMT protocol (UT IRB# HSC-SPH-13-0119). Many of the published studies have used bacteria isolated from fresh fecal samples with collection and processing on the day of planned FMT. Our currently approved study and another published study [10] have successfully used a bacterial solution obtained from filtered human feces from healthy donors that were frozen for storage at -80 °C until required for use. Furthermore, detailed microbiological studies with 16S rRNA gene sequencing demonstrated stable “engraftment” or “implantation” of donor microbiota with frozen bacterial product [11], with dramatic shifts in recipient gut microbial communities noted after transplantation.

The route of the FMT administration can be introgastric, naso-duodenal, transcolonoscopic, or enema based on previous studies. Retention (1-3 hours) enema administration of intestinal bacteria is effective, cheap, and safe and carries less procedural or institutional admission costs. The enema approach has been successfully carried out with more than 100 subjects with RCDAD [12, 13]. In addition, a recent report from Louie (Presentation IDSA, San Francisco, CA 2013) indicated that an oral capsule of concentrated intestinal bacteria successfully treated 30 of 31 subjects with RCDAD. After follow-up for 90 days, ~90% of subjects were cured from RCDAD. One patient had recurrence of diarrhea after treatment of urinary tract infection.

In our currently approved study (HSC-SPH-13-0119), we are studying subjects with recurrent CDAD with fresh, frozen or lyophilized bacterial suspension in a randomized allocation 1:1:1 via colonoscopy. So far

43 subjects with more than 3 RCDAD underwent FMT. Overall, with the 3 different forms of FMT (fresh, frozen, or lyophilized), all have shown equivalent efficacy and normalization of intestinal flora. One subject developed sepsis from a urinary tract infection complicated by cecal volvulus the week after FMT requiring surgery that was felt to be unrelated to FMT. A second subject developed *C. difficile* negative colitis a week after FMT. This is not a known complication of FMT or R CDAD.

Whether intestinal bacteria suspension from a healthy donor is fresh, frozen or lyophilized does not appear to be an important variable in terms of providing healthy bacteria to repopulate the lower intestine of a patient with depleted flora (Jiang Z-D, DuPont HL et al. unpublished data to be presented orally at the national meeting of the American College of Gastroenterology, October 2014). Also, the route of administration of the bacteria does not appear to be important with oral (Louie et al. cited above), nasoduodenal [14], colonic [15] and rectal administration [12] all being effective in successfully treating RCDAD in previous studies. Frozen donor intestinal bacteria can be stockpiled in a freezer from selected and screened donors for treatment of subjects with RCDAD as they are identified without requiring that a single donor be screened and processed the day of use for each treated patient. If effective in treating RCDAD, lyophilized intestinal bacteria can be easily and inexpensively be used for outpatient treatment by oral administration by capsules to subjects with RCDAD. Using FMT by retention enema or by capsule formulation will dramatically reduce the cost of the FMT procedure by eliminating the need for endoscopic methods for treatment.

2. OBJECTIVE

We will generate frozen or lyophilized FMT intestinal bacteria inocula from well-screened healthy volunteer donors providing ≥ 150 grams of stool/FMT. Donor intestinal bacteria suspensions will be diluted ≥ 750 mL to freeze before rectal enema procedure and diluted ≥ 300 mL for later lyophilization and encapsulation. This study will evaluate the safety of a frozen or lyophilized inoculum administered, respectively, by retention enema or capsules.

Fecal samples from donors and recipients will be saved for future studies.

3. STUDY DESIGN

This is a single center, randomized, parallel assignment, open label safety study conducted in subjects with RCDAD. Approximately 100 subjects will be enrolled in the study and randomized at 1:1 ratio to receive frozen filtered intestinal bacteria via retention enema or lyophilized donor intestinal bacteria. All subjects will be followed for approximately 6 months following FMT.

3.1 Study Structure

Donors will be enrolled and screened at the laboratory in the Center for Infectious Diseases at University of Texas School of Public Health (CID-UT-SPH) which is a College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA) certified laboratory.

Recipient subjects may self-refer but must have a physician who agrees to accept care of the patient following FMT. Subjects consenting to treatment at Baylor St. Luke's Medical Center and the University of Texas School of Public Health must be willing to self-pay for the FMT with a money order or cashier's check, or debit/credit card in the amount of \$1,500. There will be no insurance accepted. Subjects undergoing retention enema will be treated as outpatients at either Baylor St. Luke's Medical Center in the Texas Medical Center, Kelsey-Seybold Clinic - Main Campus on Holcombe Blvd., or at the Digestive Disease Center at Memorial Hermann in the Texas Medical Center. All subjects taking capsules with lyophilized intestinal bacteria suspension will be seen at Kelsey-Seybold Clinic – Main Campus on Holcombe Blvd. to receive the oral FMT. Once the procedure is completed, the recipient's care will be returned to their physician.

Evaluation of the safety of FMT and recurrence of clinical symptoms of *C. difficile* will be performed by the FMT staff. The primary endpoint is to evaluate the safety of FMT by rectal or oral routes with secondary endpoint related to efficacy, prevention of CDAD recurrence and improvement of diversity of colonic flora following receipt of FMT with either frozen filtered intestinal bacteria or lyophilized donor intestinal bacteria.

3.2 Study Population

We demonstrated in a recent study that a majority of Houston gastroenterologists and infectious diseases specialists supported the creation of an intestinal microbiota transplantation center in Houston [16]. A letter about the study will be sent to the physicians in the Houston area to recruit CDAD subjects. We will screen up to 125 recipients to obtain 100-treated subjects.

Screened volunteer donors from the Texas Medical Center area will donate stools for use for up to 100 subjects. The donors will be compensated \$10 cash for each stool donation of sufficient volume/weight. Donors may provide stool for more than one patient. The donors will be screened for health history and conditions, medication use, sexual behavior, travel, and other risk factors for infectious diseases during a private and confidential interview. All fecal material from donors will be screened for enteric pathogens. Donors will be notified in the event an infection is identified that disqualifies them from donating.

3.3 Study Procedures

3.3.1 Informed consent

Written informed consent must be obtained from each recipient or donor before he/she undergoes any procedures required by this protocol, including screening procedures.

3.3.2 Identification number

Recipient: Each patient who signs an informed consent form will be assigned a study number at CID-UT-SPH. The study number will consist of the letter „FMT-R-02-XXX“ followed by a 3-digit identification number (e.g., FMT-R-02-001). Recipients need to meet the inclusion and exclusion criteria (listed below in table).

Donor: Each donor who signs an informed consent form will be assigned a screening number at the CID-UT-SPH. Subjects must be screened before they can become a qualified donor. The screening number will consist of the letter „FMT-D-02-XXX“ followed by a 3-digit identification number (e.g., FMT-D-02-001). Donors will provide stool volume to CID within 2 hours after passing. The same donor may donate additional stool every weekday for three months.

3.3.3 Inclusion and exclusion criteria

| | Inclusion Criteria | Exclusion Criteria |
|------------|--|---|
| Recipients | <ol style="list-style-type: none"> 1. Male and female subjects 18 years of age or older 2. Sexually active female subjects of child-bearing potential must agree to use an effective method of birth control during the treatment and follow-up period 3. Required to sign an informed consent form 4. Subject's attending physician agrees to provide care following FMT 5. Able to follow study procedures and follow-ups. 6. Diagnosed by medical history of ≥ 3 RCDAD bouts in outpatients or ≥ 2 bouts of RCDAD in an inpatient with ≥ 2 positive fecal tests for <i>C. difficile</i> toxin 7. Received at least one course of adequate antibiotic therapy for CDAD (≥ 10 days of vancomycin, metronidazole or fidaxomicin) after the subjects last bout of CDAD | <ol style="list-style-type: none"> 1. Subjects with known neutropenia with absolute neutrophil count $< 0.5 \times 10^9/L$ 2. Evidence of toxic megacolon, fulminant colitis, gastrointestinal perforation, ileus, abdominal distension, lack of bowel sounds, fever, or hypotension 3. Known peripheral white blood cell count $> 15.0 \times 10^9/L$ or temperature $> 38.0^\circ C$ 4. Diarrhea due to definable non-CDAD pathogen 5. Post total colectomy or presence of a colostomy 6. Unable to tolerate FMT via enema for any reason 7. Requiring systemic non-<i>C. difficile</i> antibiotic therapy at the time of FMT 8. Actively taking <i>Saccharomyces boulardii</i> or other probiotic at the time of FMT 9. Need for continuing use of oral vancomycin, oral or IV metronidazole, fidaxomicin, rifaximin or nitazoxanide at the time of FMT and after FMT |

| | | |
|--------|--|---|
| | | 10. Severe underlying disease such that the patient is not expected to survive for one or more years or unstable medical condition requiring daily change in treatments |
| Donors | <ol style="list-style-type: none"> 1. Able to provide and sign informed consent 2. Able to complete and sign the donor questionnaire 3. Able to adhere to FMT stool collection requirements | <ol style="list-style-type: none"> 1. Tested positive for any of variables mentioned in the section of Screening of Donor 2. History of any type of active cancer or autoimmune disease 3. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire 4. History of gastrointestinal disorder, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or diarrhea 5. Antibiotic use or any systemic immunosuppressive agents in the 3 months prior to stool donation 6. Current or previous medical or psychosocial condition 7. Body mass index over 30 8. Presence of known diabetes mellitus 9. Travel to a developing country in past 3 months |

4. METHODOLOGIES

4.1 Recipient

4.1.1 Pre-screening

Before the FMT procedure, recipients will be screened by the investigators and/or FMT staff. A screening log will be designed to document the rationale for screening the patient (inclusion criteria) or not enrolling the patient (exclusion criteria, patient declines to participate or the consent form is not signed). Following documentations will be obtained or provided-

- Informed consent
- Patient demographics and medical history
- Description of FMT procedures for potential recipient
- Randomization – either retention enema or capsules

CDAD is defined as diarrhea (passage of ≥ 3 loose or watery stools per day for at least two consecutive 24-hour periods) together with a positive test for fecal *C. difficile* toxin. RCDAD is defined as the reappearance of diarrhea after therapy with a positive fecal toxin assay. Non-CDAD antimicrobial agents will be discontinued

before acceptance into the program. Oral vancomycin or fidaxomicin will be administered daily for at least 2-4 days (if not already taking) and then stopped 1-2 days before FMT. A fresh stool sample will be collected from the recipient before FMT and tested for *C. difficile* toxins and an aliquot (2 mL) saved at -80°C for future studies.

Demographic data and medical history will be recorded for each subject after the informed consent is signed. A fresh stool sample will be collected from a recipient before FMT, and on or about days 2, 7, 14, 30, 60 and 90 after FMT, tested for *C. difficile* toxins and an aliquot (2 mL) stored at -80°C for future studies.

4.1.2 One-Two Days Before FMT

- Stop taking vancomycin, fidaxomicin or any other antibiotic
- Stop taking any probiotics

4.1.3 Day before FMT

- Follow a clear liquid diet the entire day
- At 6:00 pm begin an approved colonoscopy prep (e.g. 1 liter of GoLyteLy or other suitable bowel prep)

4.1.4 Day of FMT

1. Continue clear liquid diet and until after FMT
2. All subjects take 4mg (2 capsules) of loperamide (Imodium) morning of FMT (can be taken after FMT if dose missed before)
3. Take routine prescription medications if needed
4. Subject will be provided aftercare instructions
5. For the recipient randomized to enema, they will present to either Baylor St. Luke's Day Medical Center, Kelsey-Seybold Clinic - Main Campus on Holcombe Blvd, or at the Digestive Disease Center at Memorial Hermann at the assigned time. FMT bacteria (750 mL) will be placed in a 1.5 L enema bag provided by the hospital. It will be allowed to warm and then be placed into the rectum over less than 4 hours. The FMT will be allowed to flow through the tubing to minimize the air infused into the rectum. The subject will be instructed to lie down on their back, or side. The subject's head will be placed on a pillow and a thick absorbent pad or towel placed under the lower abdomen and buttocks. The nurse will then ask the subject to pull their legs while lying on their back or side with knees bent. The nurse will insert the lubricated enema tip 3 to 4 inches into the rectum. The bag should be affixed to a hook 18-24 inches above the patient's anus. The recipient will be kept in trendelenburg (feet and buttocks ~ 10 inches higher than head) or side position with FMT infused at a rate of 2-4 ounces (±60mL) per minute. The total volume will be

administered in approximately 5-10 minutes. During the first 30 minutes of the FMT, if possible the abdomen should be massaged slowly and deeply by the nurse in a counter-clockwise direction as tolerated by the subject, to facilitate movement of the FMT in the colon. The enema flow should be temporarily stopped if subject complains of cramping and the subject should be asked to take quick, shallow breaths, resuming the flow when the discomfort passes. At completion the tube is clamped off and the nozzle removed. During and after the infusion the patient should lie on his or her back for 30 minutes, then rotate to their right side for approximately 30 minutes and then on their left side for approximately 30 minutes followed by any remaining time returning to their back. If the patient does not tolerate one or more positions they can find a comfortable position for the duration of the procedure. The preferred treatment is to continue rotating in the positions without having a bowel movement for up to 3 hours. After 1-3 hours the patient is allowed to have a bowel movement if they wish and to go home with instructions on diet and suggestion that they avoid antibiotics in the absence of clear indications. The subject will be told that Dr. DuPont is willing to talk with their physician about the safest antibiotics to use in the case they develop a treatable infection.

The recipient randomized to receive FMT capsules will be treated at the Kelsey-Seybold Clinic – Main Campus on Holcombe Blvd. Subjects will be given 100 grams of PRIM-DJ2727 in capsule form to be taken over a period of up to three hours remaining for one hour after ingesting the capsules to make sure the capsules have not been expelled by vomiting. If capsules are expelled within one hour of ingestion and can be seen in vomitus, the expelled capsules may be retaken slowly after waiting thirty minutes. The subject's chart should be documented with how many capsules were expelled, and how many were retaken if any.

Recipient will be provided with a second dose of 100 grams of PRIM-DJ2727 capsules of the same lot # to be self-administered on next day. The capsules will be kept by the recipient at 4 degrees centigrade refrigerated until taken the next day. Recipients receiving the capsules will be instructed to take the FMT capsules with water during the morning hours. Recipients are not required to fast.

4.1.5 Approximately One day after FMT

The recipients receiving the capsules will be contacted in the afternoon hours of the same day to inquire about any concerns. The recipient receiving the enema will be contacted by the study staff on or about the day after FMT. The following will be reviewed with subject:

- The FMT staff will review the recipient's diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages.

- Identify severity of any symptoms or any adverse experience
- Identify any significant medication name or dosage changes

4.1.6 Approximately Two days after FMT

- A fresh stool sample will be collected from the recipient to test for *C. difficile* toxins and an aliquot (2 mL) will be stored at -80°C for future studies. If the subject is from outside Houston area:
 - Instructions are given for shipping stool samples
 - A FedEx shipping label and container will be provided
 - Subject purchases dry ice to pack into the provided container with the sample
 - Subject calls FedEx to pick up the package.

4.1.7 Approximately Seven days after FMT

The recipient will be contacted by the study staff on or about 7 days after the FMT. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages
- Identify severity of any symptoms or any adverse experience
- Identify any significant medication name or dosage changes
- A fresh stool sample will be collected from the recipient to test for *C. difficile* toxins and an aliquot (2 mL) will be stored at -80°C for future studies.
- If the subject is from outside Houston area:
 - Instructions are given for shipping a sample
 - A FedEx shipping label and container will be provided
 - Subject purchases dry ice to pack into the provided container with the sample
 - Subject calls FedEx to pick up the package.

4.1.8 Approximately Fourteen days after FMT

The recipient will be contacted by the study staff on or about 14 days after the FMT. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages
- Identify severity of any symptoms or any adverse experience
- Identify any significant medication name or dosage changes

- A fresh stool sample will be collected from the recipient to test for *C. difficile* toxins and an aliquot (2 mL) will be stored at -80°C for future studies.
- If the subject is from outside Houston area:
 - Instructions are given for shipping a sample
 - A FedEx shipping label and container will be provided
 - Subject purchases dry ice to pack into the provided container with the sample
 - Subject calls FedEx to pick up the package.

4.1.9 Approximately Thirty days after FMT

The recipient will be contacted by the study staff on or about 30 days after the FMT. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages
- Identify severity of any symptoms or any adverse experience
- Identify any significant medication name or dosage changes
- A fresh stool sample will be collected from the recipient to test for *C. difficile* toxins and an aliquot (2 mL) will be stored at -80°C for future studies.
- If the subject is from outside Houston area:
 - Instructions are given for shipping a sample
 - A FedEx shipping label and container will be provided
 - Subject purchases dry ice to pack into the provided container with the sample
 - Subject calls FedEx to pick up the package.

4.1.10 Approximately Sixty days after FMT

The recipient will be contacted by the study staff on or about 60 days after the FMT. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages
- Identify severity of any symptoms or any adverse experience
- Identify any significant medication name or dosage changes

- A fresh stool sample will be collected from the recipient to test for *C. difficile* toxins and an aliquot (2 mL) will be stored at -80°C for future studies.
- If the subject is from outside Houston area:
 - Instructions are given for shipping a sample
 - A FedEx shipping label and container will be provided
 - Subject purchases dry ice to pack into the provided container with the sample
 - Subject calls FedEx to pick up the package.

4.1.11 Approximately Ninety days after FMT

The recipient will be contacted by the study staff on or about 90 days after the FMT. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages
- Identify severity of any symptoms or any adverse experience
- Identify any significant medication name or dosage changes
- A fresh stool sample will be collected from the recipient to test for *C. difficile* toxins and an aliquot (2 mL) will be stored at -80°C for future studies.
- If the subject is from outside Houston area:
 - Instructions are given for shipping a sample
 - A FedEx shipping label and container will be provided
 - Subject purchases dry ice to pack into the provided container with the sample
 - Subject calls FedEx to pick up the package.

4.1.12 Safety data

Participants will be contacted on or about 6 months following FMT in order to monitor any health effects for safety.

4.2 Donor

Project investigators/coordinator from the University of Texas School of Public Health will be responsible for donor identification. They will walk the donor with a FMT number and date of birth to Baylor St. Luke's Medical Center Blood Center. An employee from blood center will draw blood from the donor. Donor will be notified in the event an infection that disqualifies them is identified.

4.2.1 Pre-screening

We will pre-screen up to 20 potential donors to recruit up to 15 qualified donors. Identified donors will be found from various students and employees in the Texas Medical Center. Before stool is used for transplantation, the donor will be screened for infectious diseases using the following tests.

Stool will be collected in a semi-sterilized container. Stool needs to be delivered to the Center for Infectious Diseases laboratory at University of Texas School of Public Health within 8 hours of collection. Every fecal donation will be tested for enteric pathogens, including *Shigella*, *Salmonella*, *Campylobacter*, Shiga-toxin Producing *Escherichia coli*, and *Clostridium difficile* Toxin A/B and Norovirus.

Blood samples from the donor will be collected with a sterilized needle (25mL blood will be collected) and tested for conditions screened for blood transfusions the items listed below:

1. Hepatitis B core Antibody
2. Hepatitis B Surface Antigen
3. Hepatitis C virus Antibody
4. HIV-1 and HIV-2 Antibody
5. Anti-HTLV I/II
6. Serologic Test for Syphilis

4.2.2 One day before donation

- Study staff will contact qualified donor to provide ≥ 150 grams of stool on the day of FMT
- Donor is encouraged to take one stool softener laxative the evening before the procedure and drink of plenty water.
- Study staff will provide the supplies and instruction for sample collection.

4.2.3 Day of donation – preparation, storage and shelf life

Donor Stool:

- ≥ 150 g of stool per transplant (all stools < 150 grams will be discarded). The fresh stool must be brought to the laboratory within 2 hours for processing.
- Additional stool can be donated each weekday for 3 months being compensated \$10 for each donation.

Preparing Stool for FMT:

- Mix the stool sample with sterilized 0.9% NaCl without antibacterial preservative (750mL, 1:5 dilution).
- Mix above solution in a homogenizer mixer with sterilized bag (Stomacher). Initially, use the low setting until the sample breaks up, and then advance the speed gradually to the highest setting. Continue for 2 – 4 minutes until sample is smooth and homogeneous.
- For frozen intestinal bacteria - the suspension will be filtered using a sterile paper coffee filter (or gauze) that has been moistened with saline and funnel (both are sterilized). Allow adequate time for slow filtration to end. NOTE: To expedite process, multiple funnel/filters can be used to combine product.
 - Repeat above step. NOTE: it may take 2 hours to complete filtration.
 - Place filtered suspension of bacteria (750 mL) into a 1.5 L enema bag provided by nursing personnel at the clinical facility (St. Luke's/Kelsey-Seybold/Memorial Hermann)
 - Number the samples as FMT-D-02-XXX-Frozen Intestinal Bacteria
 - Label the tubes with lot number (mm-dd-yyyy) and date and time processed
- For lyophilized intestinal bacteria - the suspension will be filtered using a sterile paper coffee filter (or gauze) that has been moistened with saline and funnel (both are sterilized). Allow adequate time for slow filtration to end. NOTE: To expedite process, multiple funnel/filters can be used to combine product.
 - Repeat above step. NOTE: it may take 2 hours to complete filtration.
 - Place filtered suspension in 15 sterilized 50 mL tubes
 - Number the samples as FMT-D-02-XXX-Lyophilized Intestinal Bacteria
 - Label the tubes with lot number (mm-dd-yyyy) and date and time processed
 - Freezing at -80°C overnight: The product (filtered intestinal bacteria in 50mL tube) is frozen
 - After freezing, the product is placed under vacuum. This enables the frozen solvent in the product to vaporize without passing through the liquid phase, a process known as sublimation.
 - A lyophilized product must be sealed with parafilm within its container, then will be taken to Compounding Shop Pharmacy 11851A Wilcrest, Houston, TX to produce enteric coated capsules
- Storage Condition of FMT Products -
 - FMT-D-02-001-Frozen bacteria from ≥ 150 g stool sealed with plastic (polyethylene or similar film) at -80°C in room 804 RAS (CID-0066-CAP) for up to 12 months in a test tube rack clearly labelled with the lot # and expiration date
 - FMT-D-02-001-Lyophilized Capsules containing bacteria from ≥ 150 g stool will be kept in a sterilized vial at 4°C in room 740 RAS (CID-0068-CAP) for up to 12 months in a test tube rack clearly labelled with the lot # and expiration date
- Upon completion of the process, stool samples must recorded into the Donor Specimens Log.

- If necessary, we will use repeat stool donors who are perceived to be safer than first time donors. If the repeat donor stool is used within 3 months of passing the questionnaire survey, no blood and stool will be screened. If the donation is 3 months over the before mentioned process, then the medical history must be negative and both blood and stool will be tested for the items detailed above. We are using the 3 months" time for repeat screening, as this is what is being used in an NIH sponsored study at Emory University School of Medicine. We feel that this is adequate when combined with an unchanged medical history to assure safe FMT material.

5. EVALUATION OF SAFETY

5.1 Primary Safety Endpoints

The following safety endpoints will be evaluated in this study.

5.1.1 Medical conditions

When contacting subjects on or about days 1, 7, 14, 30, 60 and 90 after FMT, we will review the recipient diary with the recipient to review any adverse experience or medication change since the medical history obtained at time of FMT. The data will be recorded accurately and completely in the recipient diary. In order to monitor any health effects for safety, participants will be contacted on or about 6 months following FMT.

Investigators should determine if any adverse experience or medication need to be further studied. A letter and reference publication will be sent to recipient's primary physician if he/she develops RCDAD after FMT. Donor will be contacted and tested if it is necessary. All actions will be recorded with medical condition, dates of adverse experience and medication taken, indication for new medication taken, and total daily dose.

Recipients should be instructed at FMT to contact the study staff if they have any questions regarding adverse experience or the appropriateness of a medication after FMT.

5.1.2 Monitoring for safety

The following definitions of terms are guided by the International Conference of Harmonization and US Code of Federal Regulations (21 CFR 312.32).

An adverse experience is any unfavorable or unintended sign, symptom, of disease temporally associated with FMT procedure, whether or not considered related to the procedure, including, but not limited to:

- Any symptom not previously reported by the recipients (Medical history)
- An exacerbation of a pre-existing illness, increases in the frequency and/or severity of the signs or symptoms of CDAD (defined as positive *C. difficile* toxin test and enteric symptoms)
- A significant increase in frequency or intensity of a pre-existing episodic event or condition

- A condition first detected or diagnosed after study drug administration even though the condition may have been present before the procedure

Details of all adverse experiences that occur after FMT will be collected as indicated above.

Serious adverse experience is any adverse experience that:

- Results in death
- Is life threatening (at immediate risk of death from the procedure as it occurred)
- Requires inpatient hospitalization (overnight stay) or prolongs a current hospitalization
- Causes a persistent or significant disability/incapacity
- Of medical importance (any event that requires medical or surgical intervention to prevent one of the outcomes listed above)

The investigator will exercise medical and scientific judgment when deciding whether expeditious reporting is appropriate in other situations not strictly meeting the listed criteria above. The investigators will meet/discuss with experts in the field if there is a question of whether the adverse experience would be considered serious.

Severity – The adverse experience will be documented according to the following descriptors:

- *Mild*: associated with no limitation of usual activities or only slight discomfort
- *Moderate*: associated with limitation of usual activities or significant discomfort
- *Severe*: associated with inability to carry out usual activities or very marked discomfort

Relationship – the relationship of adverse experience to FMT will be assigned by the Investigator according to the following definitions:

- *Probable*: a reaction that follows a reasonable temporal sequence from the procedure that follows a known or expected response pattern to the suspected procedure and that could not be reasonably explained by the known characteristics of that patient's clinical state
- *Possible*: a reaction that follows a reasonable temporal sequence from the procedure that follows a known or expected response pattern to the procedure but could readily have been produced by a number of other factors

- *Unlikely*: a reaction that does not follow a reasonable temporal sequence from the procedure but for which causality from FMT cannot be ruled out.
- *Not related*: a reaction for which sufficient data exist to indicate that the etiology is unrelated to the procedure

5.1.3 Pre-existing signs and symptoms and medical conditions

Medical conditions that are present at or before the procedure that manifest with the same severity or frequency will not be recorded as adverse experience. Similarly, signs or symptoms related to a pre-existing disease will not be recorded as adverse experience unless there is an increase in the severity or frequency of the signs or symptoms. These pre-existing conditions, signs, or symptoms will be recorded on the Medical History.

5.1.4 Progression of underlying conditions as an adverse experience

If the progression of the underlying condition might be reasonably anticipated given the nature and severity of the underlying condition, then the progression of the underlying condition *per se* will not constitute an adverse experience. However, if the progression of the underlying condition is fatal, then the progression of the underlying condition should be reported as an adverse experience.

5.1.5 Recording and documenting adverse experience

The Investigator must completely and promptly record each new adverse experience and serious adverse experience that occurs after the procedure, even if the relationship of adverse experience to the procedure is assessed by the Investigator to be “unlikely” or “not related”. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. If an adverse experience meets the definition of a serious adverse experience then the Investigator must also complete the serious adverse experience, and also send any supporting source documents directly to the University of Texas Health Science Center IRB as soon as the event is discovered. At each visit, after the patient has had an opportunity to mention any problems spontaneously, the Investigator (or designee) will inquire about adverse experience by asking the standard questions listed in, such as:

- Have you had any medical problems since your last visit?
- Have any medical problems present at your last visit changed, i.e., stopped, worsened, or improved?
- Have you taken any new medicines, other than study drug, since your last visit?

Any spontaneous adverse experience information provided by the patient will be recorded.

5.1.6 Investigator reporting of serious adverse experience

All serious adverse experience must be reported to the University of Texas Health Science IRB using the serious adverse experience by facsimile or email or by telephone as soon as the serious adverse experience is discovered, and within 24 hours after the Investigator recognizes or classifies the event as a serious adverse experience. A brief description of the event must be provided at the time of the initial serious adverse experience report. The initial serious adverse experience report should be followed up by additional information using the serious adverse experience within 48 hours. The reports should identify the patient by their unique patient number instead of names. The completed serious adverse experience form will be used by the investigators in regulatory filings. The investigator is responsible for continuing to report to the University of Texas Health Science IRB any new or relevant follow-up information obtained concerning the serious adverse experience. The results of any additional assessments conducted must be also reported to the University of Texas Health Science IRB.

5.1.7 Notification of post-study serious adverse experience

Investigators are not obligated to actively seek follow-up information for subjects with adverse experience after the conclusion of the study. However, if the investigator becomes aware of an adverse experience that occurs after the patient completes and the adverse experience is considered by the Investigator to be at least possibly related to study procedure, the investigator must notify the University of Texas Health Science Center IRB.

5.2 Secondary Efficacy Endpoint

The secondary endpoint is failure to have a subsequent bout of CDAD in 60 days post FMT. It is defined as recipient has had another episode of diarrhea (≥ 3 unformed stools per 24-hours for at least two consecutive days) with positive *C. difficile* toxin A/B test. An unformed stool is defined as either a soft or watery stool. Data will be recorded in the CRFs. Subjects will receive FMT up to three times within the study treatment time of 90 days after each FMT. After the third FMT no further will be performed, and if subject develops another recurrence of *C. difficile*, they will be returned to the care of their physician.

6. DATA COLLECTION AND ANALYSIS

6.1 Recording of Data

The Investigator must maintain adequate and accurate source documents. The source documents will be reviewed and signed at the investigative site by the investigators periodically.

6.2 Statistical Methods

6.2.1 General considerations

All analyses will be performed and all tables, figures, and data listings will be prepared using SAS (Cary, NC). Summary statistics for continuous variables will include the mean, standard deviation, median, minimum, and maximum value; categorical variables will be presented as counts and percentages. For all analyses, baseline is defined as the date of the transplantation.

6.2.2 Power calculations

No power calculation was performed since this is a pilot study. We will evaluate the program/center after enrollment of up to 100 recipients with RCDAD. Hundred subjects (50 in each group, receive either frozen or lyophilized intestinal bacteria from healthy donors) with RCDAD will be included in the study using the entry criteria in section 4.3.3.

6.2.3 Analysis populations

All analyses will be conducted using the intent-to-treat population. Intent-to-treat is defined as all recipients who have started the procedure.

Listings and summary tables with descriptive statistics will be prepared for demographic data (gender, date of birth), and medical history data (including the date and time of onset of CDAD and signs or symptoms of enteric infection, episodes of RCDAD).

6.2.4 Randomization procedures

An independent statistician will develop a randomization list using SAS. Randomization in permuted blocks will be used to achieve balance across treatment groups. The randomization scheme consists of a sequence of blocks such that each block contains a pre-specified number of treatment assignments in random order. The purpose of this is to balance the randomization scheme at the completion of each block. The target sample size is 100 recipients (50 receive frozen and 50 receive lyophilized intestinal bacteria). Subject study identification assignment log will be completed and kept in the study binder.

6.2.5 Analyses of safety

All adverse experience and serious adverse experience will be coded using the Medical Dictionary for Regulatory Activities Version 12.0 which is a registered trademark of the International Federation of Pharmaceutical Manufacturers and Associations. The number and percentage of subjects reporting at least one occurrence of adverse experience and/or serious adverse experience for each system organ class and preferred term will be tabulated. Term will also be tabulated by severity and by the relationship to the procedure. For multiple occurrences of the same adverse experience with different severities, the adverse experience with the highest severity will be tabulated. For multiple occurrences of the same adverse experience with different relationships to the procedure (related and not related); the adverse experience will be tabulated as related. All adverse experience and or serious adverse experience for all subjects will be presented in data listings. Narratives will also be prepared for each serious adverse experience.

6.2.6 Secondary efficacy analyses

The proportion of subjects in each group (frozen and lyophilized product) achieving Clinical Cure will be compared using the Chi-square test. The proportion of recipients with a subsequent bout of CDAD in 60 days post FMT in each treatment group will be compared using the Chi-square test.

7 PROTECTING PRIVACY

This study protocol, documentation data, and all other information generated, will be held in strict confidence by the Investigators. No information concerning the study or the data will be released to any unauthorized third party without prior written approval by the investigators.

8 CONTACTS

- Dr. Herbert L. DuPont at (832) 877-0885 for Baylor St. Luke's and Kelsey-Seybold Clinic transplants/pills
- Dr. Andrew W. DuPont at (281) 703-1650 for Memorial Hermann transplants
- Dr. Zhi-Dong Jiang at (832) 380-5183 for donor screening issues
- The University of Texas Health Science Center Committee for the Protection of Human Subjects at (713) 500-7942
- The Baylor St. Luke's Episcopal Hospital Institutional Review Board at (832) 355-3347

9 REFERENCES

- [1] C.P. Kelly, J.T. LaMont, *Clostridium difficile*--more difficult than ever, *The New England journal of medicine*, 359 (2008) 1932-1940.
- [2] D. Gravel, M. Gardam, G. Taylor, M. Miller, A. Simor, A. McGeer, J. Hutchinson, D. Moore, S. Kelly, M. Mulvey, P. Canadian Nosocomial Infection Surveillance, Infection control practices related to *Clostridium difficile* infection in acute care hospitals in Canada, *American journal of infection control*, 37 (2009) 9-14.
- [3] J.Y. Chang, D.A. Antonopoulos, A. Kalra, A. Tonelli, W.T. Khalife, T.M. Schmidt, V.B. Young, Decreased Diversity of the Fecal Microbiome in Recurrent *Clostridium difficile*-Associated Diarrhea, *J Infect Dis*, 197 (2008) 435-438.
- [4] M. Tvede, J. Rask-Madsen, Bacteriotherapy for chronic relapsing *Clostridium difficile* diarrhoea in six patients, *Lancet*, 1 (1989) 1156-1160.
- [5] A. Khoruts, J. Dicksved, J.K. Jansson, M.J. Sadowsky, Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea, *J Clin Gastroenterol*, 44 (2010) 354-360.
- [6] F. Rohlke, C.M. Surawicz, N. Stollman, Fecal flora reconstitution for recurrent *Clostridium difficile* infection: results and methodology, *Journal of clinical gastroenterology*, 44 (2010) 567-570.
- [7] S.S. Yoon, L.J. Brandt, Treatment of refractory/recurrent *C. difficile*-associated disease by donated stool transplanted via colonoscopy: a case series of 12 patients, *Journal of clinical gastroenterology*, 44 (2010) 562-566.
- [8] M.S. Silverman, I. Davis, D.R. Pillai, Success of self-administered home fecal transplantation for chronic *Clostridium difficile* infection, *Clin Gastroenterol Hepatol*, 8 (2010) 471-473.
- [9] H.L. Dupont, Diagnosis and Management of *Clostridium difficile* Infection, *Clin Gastroenterol Hepatol*, 11 (2013) 1216-1223.
- [10] M.J. Hamilton, A.R. Weingarden, M.J. Sadowsky, A. Khoruts, Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium difficile* infection, *The American journal of gastroenterology*, 107 (2012) 761-767.
- [11] M.J. Hamilton, A.R. Weingarden, T. Unno, A. Khoruts, M.J. Sadowsky, High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria, *Gut microbes*, 4 (2013) 125-135.
- [12] Z. Kassam, R. Hundal, J.K. Marshall, C.H. Lee, Fecal transplant via retention enema for refractory or recurrent *Clostridium difficile* infection, *Arch Intern Med*, 172 (2012) 191-193.
- [13] F. Rohlke, N. Stollman, Fecal microbiota transplantation in relapsing *Clostridium difficile* infection, *Therapeutic advances in gastroenterology*, 5 (2012) 403-420.
- [14] E. van Nood, A. Vrieze, M. Nieuwdorp, S. Fuentes, E.G. Zoetendal, W.M. de Vos, C.E. Visser, E.J. Kuijper, J.F. Bartelsman, J.G. Tijssen, P. Speelman, M.G. Dijkgraaf, J.J. Keller, Duodenal infusion of donor feces for recurrent *Clostridium difficile*, *N Engl J Med*, 368 (2013) 407-415.
- [15] L.J. Brandt, S.S. Reddy, Fecal microbiota transplantation for recurrent *clostridium difficile* infection, *J Clin Gastroenterol*, 45 Suppl (2011) S159-167.
- [16] Z.D. Jiang, L.N. Hoang, T.M. Lasco, K.W. Garey, H.L. Dupont, Physician attitudes toward the use of fecal transplantation for recurrent *Clostridium difficile* infection in a metropolitan area, *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 56 (2013) 1059-1060.